Expanded response surface model for predicting the effects of temperatures, pH, sodium chloride contents and sodium nitrite concentrations on the growth rate of *Yersinia enterocolitica*

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S. BHADURI, R.L. BUCHANAN AND J.G. PHILLIPS. 1995. The previously reported data set for the low temperature (5, 12 and 19°C) of *Yersinia enterocolitica* was expanded to include higher abusive temperature (28, 37 and 42°C). In addition to temperature, the data set included the effects and interactions of pH (4·5–8·5), sodium chloride (0·5–5%) and sodium nitrite (0–200 $\mu g\ ml^{-1}$) on the aerobic growth of *Y. enterocolitica* in brain heart infusion broth. Growth curves were modeled by fitting viable count data to the Gompertz equation. Quadratic models of natural logarithm transformations of the Gompertz B and M values and the derived values for lag phase durations and generation times were obtained using response surface analyses. Predictions based on the models for B and M values were comparable to predictions based on the derived values. These revised models provide an expanded means for rapidly estimating how the bacterium is likely to respond to any combination of the four variables within the specified ranges.

INTRODUCTION

Yersinia enterocolitica is an enteric pathogen that has been implicated in a number of outbreaks of gastrointestinal disease (Doyle and Cliver 1990; Lee *et al.* 1990; Kapperud 1991). This psychrotroph is able to multiply in refrigerated foods (Gill and Reichel 1989; Doyle and Cliver 1990; Kapperud 1991). The micro-organism has been isolated from a variety of different food types, including vegetables, dairy products (including dry milk products), and pork (Doyle and Cliver 1990; Lee *et al.* 1990; Kapperud 1991), though not all of these isolates contain the virulence-associated plasmid. Control of this foodborne pathogen both in refrigerated foods and foods kept at abusive temperatures requires a clear understanding of the interaction of temperatures with secondary barriers such as pH, water activity or atmosphere composition. This knowledge is needed to permit better estimates of the shelf-life of products that will ensure use before psychrotrophic pathogens such as *Y. enterocolitica* have had an opportunity for significant growth.

This need to quantitatively consider multiple barriers can be achieved through recent advances in predictive microbiology. A number of models relating to the growth of *Y. enterocolitica* at sub-optimal temperatures (0–25°C) and/or pH levels (4·5–6·5) in the presence of different acidulants have been described (Adams *et al.* 1991; Alber and Schaffner 1992; Little *et al.* 1992a,b). Using spectrophotometric techniques, Hudson (1993) developed response surface models for the growth of two *Y. enterocolitica* strains as a function of temperature, pH, sodium chloride and sodium nitrite. Recently, this group (Bhaduri *et al.* 1994) developed an effective response surface model based on viable counts for growth of *Y. enterocolitica* under combined effects of low temperature (5–19°C), pH (4·5–7·5), sodium chloride (0·5–5%) and sodium nitrite (0–200 $\mu g\ ml^{-1}$). While there has been a great deal of interest in modeling *Y. enterocolitica* growth at low temperatures, little systematic information is available on the growth characteristics of *Y. enterocolitica* at abusive temperatures. Thus, as part of the continued interest in characterizing growth of

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Y. enterocolitica in foods, this group studied the effects of pH, sodium chloride and sodium nitrite on growth of *Y. enterocolitica* at abusive temperatures (28–42°C). The current study presents these data and their integration with previous data gathered at low temperatures to generate a more comprehensive model for the growth of *Y. enterocolitica*.

MATERIALS AND METHODS

Bacteria

Three plasmid-bearing virulent strains, GER (Serotype O : 3), PT18-1 (Serotype O : 5, O : 27), and WA (Serotype O : 8), of *Y. enterocolitica* were kindly provided by W.E. Hill, Food and Drug Administration. Avirulent plasmidless isogenic derivatives were obtained by isolating large, flat white colonies which emerged when plasmid-bearing cultures were grown at 37°C on Congo red brain heart infusion agarose as described by Bhaduri *et al.* (1991). These isogenic plasmidless strains were used because they grow faster than their corresponding plasmid-bearing virulent strain, thereby insuring an appropriately conservative model for the pathogen. These were the same strains employed to produce the original model that covered only the lower temperature range (Bhaduri *et al.* 1994).

Culture conditions

The three strains were cultured separately in 10 ml test tubes containing 5 ml of Brain Heart Infusion broth (BHI) (Difco Laboratories, Detroit, MI) for 18–24 h at 28°C. Each strain grew to a population density of approximately 10^{10} cfu ml^{-1}. Equal volumes of the three cultures were combined. The mixed culture was then diluted using 0·1% peptone water, and used to inoculate the experimental media. The target inoculum level was 2×10^3 cfu ml^{-1}.

The culture techniques employed were identical to those described by Buchanan *et al.* (1989). The culture medium used was BHI broth modified to have the pH and sodium chloride levels described in Table 1. Basal BHI medium contains 0·5% NaCl (w/v) and has a pH of 7·3. This was modified by adjustment of pH using HCl or NaOH and by addition of appropriate amounts of crystalline NaCl. Triplicate 50-ml portions of medium were dispensed in 250 ml Erlenmeyer flasks and autoclaved for 20 min at 120 psi. After cooling, the desired concentration of $NaNO_2$ was added as a filter-sterilized stock solution. After inoculation with 0·1 ml of the diluted mixed culture, the flasks were incubated on rotary shakers (160 rev min^{-1}) at the different experimental temperatures.

Variables and experimental design

The following variables were studied in conjunction with a partial factorial design for four variables: temperature (28, 37, 42°C), pH (4·5–8·5, in 1·0-pH unit increments), NaCl (0·5–5%, in 1·5% increments), and $NaNO_2$ (0–200 μg ml^{-1} in 50-μg ml^{-1} increments).

Bacteriology

At appropriate intervals, samples were removed, and diluted as needed in 0·1% peptone water and immediately surface plated with a Spiral Plater (Model D, Spiral Systems, Bethesda, MD) on duplicate BHI agar (Difco) plates. After 24 h incubation at 37°C, colonies were counted with a Laser Bacteria Colony Counter (Spiral Systems Instruments, Inc., Bethesda, MD).

Certain combinations of variables did not support growth. 'No growth' was defined as a count at the end of an incubation period of 21 d at or below the starting count.

Curve fitting

Growth curves were generated from the experimental data using the Gompertz equation as described in a previous report (Bhaduri *et al.* 1994). The four Gompertz parameters were subsequently used to calculate lag phase duration (LPD), generation time (GT), exponential growth rate (EGR), maximum population density (MPD) and 'time to a 1000-fold increase' (T_{1000}) values.

Model development

Polynomial models for the effect of temperature, pH, sodium chloride content and sodium nitrite concentration were calculated for natural logarithm (Ln) transformations of the Gompertz B and M parameters, and Ln transformations of the derived LPD and GT values calculated for individual growth curves. All models were generated using the SAS General Linear Model procedure (SAS 1989).

RESULTS AND DISCUSSION

As in a previous study (Bhaduri *et al.* 1994), the effect of inoculum size on the growth kinetics of *Y. enterocolitica*

Table 1 Growth kinetics values for *Yersinia enterocolitica* cultured under various combinations of temperature pH, NaCl content and sodium nitrite concentration

Independent variables				Observed kinetic values								T_{1000} calculated using individual C-values	T_{3000} calculated using mean C-values*
Temperature (°C)	pH	NaCl	$NaNO_2$	A	C	B	M	EGR	GT	LPD	MPD		
28	4·5	0·5	0	3·20	NG	0·0000	—	—	—	—	—	—	—
28	4·5	0·5	50	3·25	NG	0·0000	—	—	—	—	—	—	—
28	4·5	2·0	50	3·50	NG	0·0000	—	—	—	—	—	—	—
28	4·5	3·5	0	3·50	NG	0·0000	—	—	—	—	—	—	—
28	5·0	0·5	0	3·11	6·40	0·1691	9·12	0·398	0·8	3·2	9·5	10·8	10·2
28	5·5	2·0	0	3·52	6·53	0·1335	24·32	0·321	0·9	16·8	10·0	26·2	25·7
28	6·5	0·5	0	3·18	7·06	0·1817	8·06	0·472	0·6	2·5	10·2	8·9	9·1
28	6·5	0·5	200	3·27	7·27	0·1622	8·22	0·434	0·7	2·0	10·5	9·0	9·3
28	6·5	2·0	0	3·33	6·72	0·1368	10·95	0·337	0·9	3·5	10·1	12·5	12·3
28	6·5	3·5	0	3·89	5·60	0·1792	14·89	0·369	0·8	9·3	9·5	17·5	15·9
28	6·5	5·0	0	3·75	5·64	0·0732	21·59	0·151	2·0	7·9	9·4	27·9	24·1
28	6·5	5·0	200	3·65	5·92	0·0715	21·68	0·156	1·9	7·7	9·6	27·1	24·2
28	7·5	3·5	50	2·86	6·15	0·0975	17·40	0·220	1·4	7·1	9·0	20·8	19·3
28	8·5	0·5	0	3·34	6·05	0·4541	7·68	1·011	0·3	5·5	9·4	8·5	8·1
28	8·5	0·5	200	3·42	5·91	0·4310	7·10	0·932	0·3	4·7	9·3	8·0	7·5
28	8·5	3·5	0	2·53	7·33	0·1135	21·84	0·306	1·0	13·0	9·9	22·8	23·4
28	8·5	3·5	200	2·91	7·09	0·1670	28·98	0·430	0·8	22·4	10·0	29·9	30·1
37	4·5	0·5	0	2·95	NG	0·0000	—	—	—	—	—	—	—
37	5·0	0·5	0	3·51	5·87	0·1801	11·28	0·389	0·8	5·7	9·3	13·5	12·3
37	5·5	0·5	0	3·20	6·44	0·2590	12·97	0·611	0·5	9·1	9·7	14·0	13·7
37	5·5	0·5	200	3·17	6·56	0·1109	55·04	0·268	1·1	46·0	9·7	57·3	56·7
37	5·5	2·0	0	3·08	7·46	0·0438	32·28	0·119	2·6	8·8	10·5	34·4	36·4
37	5·5	3·5	0	3·55	5·91	0·2046	11·35	0·445	0·7	6·5	9·4	13·3	12·2
37	5·5	5·0	0	3·31	6·22	0·1536	27·03	0·352	0·9	20·4	9·5	29·1	28·2
37	5·5	5·0	200	3·58	5·90	0·2070	11·09	0·448	0·7	6·2	9·5	13·0	12·0
37	6·5	0·5	0	3·53	5·85	0·2194	6·64	0·472	0·6	2·1	9·4	8·5	7·5
37	7·5	0·5	0	3·46	5·99	0·3052	7·28	0·671	0·4	3·9	9·5	8·5	7·9
37	8·5	0·5	0	3·68	5·94	0·2900	6·70	0·632	0·4	3·2	9·5	8·0	7·3
37	8·5	5·0	0	3·50	NG	0·0000	—	—	—	—	—	—	—
37	8·5	5·0	200	3·10	NG	0·0000	—	—	—	—	—	—	—
42	4·5	0·5	0	3·50	NG	0·0000	—	—	—	—	—	—	—
42	4·5	3·5	0	3·25	NG	0·0000	—	—	—	—	—	—	—
42	5·5	0·5	0	3·05	5·04	0·2399	10·38	0·445	0·7	6·2	8·1	13·1	11·1
42	5·5	2·0	50	3·75	NG	0·0000	—	—	—	—	—	—	—
42	5·5	5·0	50	3·50	NG	0·0000	—	—	—	—	—	—	—
42	6·5	0·5	100	3·68	6·00	0·1215	10·77	0·268	1·1	2·5	9·7	13·8	12·3
42	6·5	2·0	0	3·59	4·93	0·2828	27·71	0·512	0·6	24·2	8·5	30·2	28·4
42	6·5	3·5	100	3·50	NG	0·0000	—	—	—	—	—	—	—
42	7·5	0·5	0	2·73	7·08	0·1193	8·69	0·311	1·0	0·3	9·8	10·0	10·2
42	7·5	2·0	200	3·75	NG	0·0000	—	—	—	—	—	—	—
42	7·5	5·0	200	3·60	NG	0·0000	—	—	—	—	—	—	—
42	8·5	0·5	0	3·69	6·11	0·2315	5·75	0·519	0·6	1·4	9·8	7·2	6·5
42	8·5	5·0	200	3·10	NG	0·0000	—	—	—	—	—	—	—

* The grand mean of all cultures from the current study and those from Bhaduri *et al.* (1994). C = 6·91, assumed.

A, Asymptotic log count of bacteria as time decreases indefinitely; C, asymptotic amount of growth that occurs as time increases; B, relative growth rate at time M; M, the time at which the absolute growth rate is maximal in hours; EGR, exponential growth rate; GT, generation time; LPD, lag phase duration; MPD, maximum population density; NG, no growth.

was examined. Inoculum levels of 10^3–10^5 cfu ml^{-1} were employed in conjunction with BHI containing 5% NaCl and 0 μg ml^{-1} $NaNO_2$ at pH 7·5 with cultures being incubated at 37°C. Little, if any, effect on the growth kinetics (LPD and GT) of *Y. enterocolitica* could be attributed to differences in inoculum levels (data not shown). This indicated that, as before, the Gompertz A-term did not require modeling.

The second supplemental analysis was an evaluation of the effect of the independent variables on the MPD. If *Y. enterocolitica* grew, it typically achieved an MPD of between 10^9 and 10^{11} cfu ml^{-1}. Only when a combination of two or more variables approached values that prevented growth was there any depression of MPD below 10^9 cfu ml^{-1}. Therefore, MPD was not modeled and C, A and MPD were estimated by the grand mean of the experimental values (6·91, 2·97 and 9·87, respectively).

A total of 126 new cultures, representing 42 variable combinations, were examined (Table 1). They were combined with the earlier data set (Bhaduri *et al.* 1994) for a total of 225 growth curves and 75 variable combinations. Both EGR and LPD were influenced by temperature, pH and NaCl. T_{1000} values increased with decreasing temperature, increasing sodium chloride levels, and acid or alkaline pH values. There was relatively little difference in T_{1000} values calculated using individual C values or the grand mean (C = 6·91) (Table 1). While growth was observed over the temperature (5–42°C), pH (4·5–8·5) and NaCl (0·5–5%) ranges tested, three combinations at low temperatures (5–19°C) had previously been reported (Bhaduri *et al.* 1994) not to support growth. Twelve additional combinations at abusive temperatures (28–42°C) in the present study also did not support growth (Table 1). As reported previously for low temperatures (Bhaduri *et al.* 1994), sodium nitrite had little effect on the growth of *Y. enterocolitica*.

The data were used to develop two sets of quadratic response surface models; one based on the natural logarithm (Ln) transformation of the Gompertz B and M parameters and the other on the Ln transformation of the LPD and GT derived from the Gompertz equation (Table 2). The R^2 values associated with the models suggest a reasonable fit between the models and the observed data. The poorest agreement was the model for LPD (R^2 = 0·787). That growth kinetic is often the most variable and thus the most difficult to successfully model. The R^2 values in the

Table 2 Second order response surface models on temperature (T) (5–42°C), pH (P) (4·5–8·5), NaCl (S) (0·5–5·0%) and $NaNO_2$ (N) (0–200 μg ml^{-1}) for aerobic growth of *Yersinia enterocolitica*

Gompertz—parameter models

B value: [1]

$$\begin{aligned}\text{Ln (B)} = &-9{\cdot}306 + 0{\cdot}1974\,T + 1{\cdot}051\,P + 0{\cdot}5411\,S - 0{\cdot}01\,N + 0{\cdot}00135\,TP \\ &- 0{\cdot}00346\,TS + 0{\cdot}0000267\,TN - 0{\cdot}0979\,PS + 0{\cdot}000941\,PN \\ &- 0{\cdot}000231\,SN - 0{\cdot}00276\,T^2 - 0{\cdot}0639\,P^2 + 0{\cdot}0173\,S^2 + 0{\cdot}000017\,N^2\end{aligned}$$

$$R^2 = 0{\cdot}879$$

M. value: [2]

$$\begin{aligned}\text{Ln (M)} = &\ 14{\cdot}699 - 0{\cdot}2233\,T - 2{\cdot}5086\,P - 0{\cdot}05034\,S + 0{\cdot}00359\,N - 0{\cdot}00225\,TP \\ &+ 0{\cdot}000301\,TS + 0{\cdot}0000624\,TN + 0{\cdot}1434\,PS - 0{\cdot}000374\,PN \\ &- 0{\cdot}000188\,SN + 0{\cdot}00358\,T^2 + 0{\cdot}1654\,P^2 - 0{\cdot}0424\,S^2 - 0{\cdot}00000485\,N^2\end{aligned}$$

$$R^2 = 0{\cdot}888$$

Derived kinetics—parameter models

Lag phase duration: [3]

$$\begin{aligned}\text{Ln (LPD)} = &\ 20{\cdot}2498 - 0{\cdot}1787\,T - 4{\cdot}515\,P - 0{\cdot}02801\,S - 0{\cdot}00183\,N - 0{\cdot}00897\,TP \\ &- 0{\cdot}00254\,TS + 0{\cdot}000153\,TN + 0{\cdot}1621\,PS + 0{\cdot}000117\,PN - 0{\cdot}000508\,SN \\ &+ 0{\cdot}00349\,T^2 + 0{\cdot}3187\,P^2 - 0{\cdot}0699\,S^2 - 0{\cdot}00000362\,N^2\end{aligned}$$

$$R^2 = 0{\cdot}787$$

Generation time: [4]

$$\begin{aligned}\text{Ln (GT)} = &\ 6{\cdot}5873 - 0{\cdot}1958\,T - 0{\cdot}982\,P - 0{\cdot}2269\,S + 0{\cdot}00689\,N - 0{\cdot}0029\,TP \\ &- 0{\cdot}00124\,TS - 0{\cdot}00000944\,TN + 0{\cdot}0653\,PS - 0{\cdot}000615\,PN \\ &+ 0{\cdot}0000346\,SN + 0{\cdot}00319\,T^2 + 0{\cdot}0643\,P^2 - 0{\cdot}00315\,S^2 - 0{\cdot}0000134\,N^2\end{aligned}$$

$$R^2 = 0{\cdot}897$$

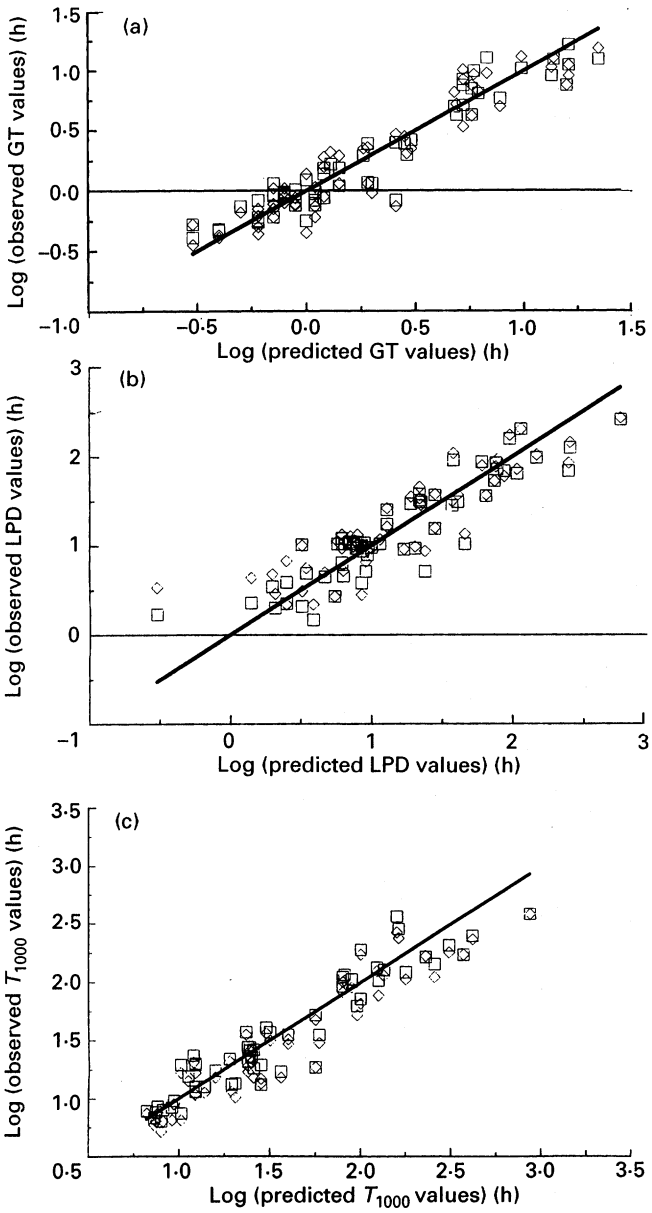

Fig. 1 Comparison of observed values for generation (GT) and lag phase duration (LPD) and 'time to 1000-fold increase in population density' (T_{1000}) with those predicted using kinetics parameters and Gompertz parameters-based models. (a) GT; (b) LPD; (c) T_{1000}. ◇, Gompertz-based model; □, kinetics-based model

current study were substantially reduced compared to earlier models for more restricted temperature range (Bhaduri *et al.* 1994). This reflects the increased difficulty in modeling growth as the optimal temperatures are reached and exceeded, and the population are subject to increased physiological stress. It is possible that better fits could have been achieved using a higher order response surface model (i.e. cubic *vs* quadratic); however, this also increases the potential for anomalous predictions within the multi-dimensional space being examined.

Table 3 F-values for independent variables and their cross products for the quadratic models based on Ln transformations

	Ln(B)	Ln(M)	Ln(LPD)	Ln(GT)
Temperature (°C)	75·3*	98·3*	23·0*	94·6*
pH	7·6**	44·3*	52·4*	8·5**
NaCl	6·8**	6·0**	0·7	1·5
$NaNO_2$	9·2**	1·2	0·1	5·5**
Temp*pH	0·2	0·7	4·2**	1·5
Temp*NaCl	3·3	0·0	0·7	0·5
Temp*$NaNO_2$	0·5	2·6	5·7**	0·1
pH*NaCl	15·4*	33·9*	15·8*	8·8**
pH*$NaNO_2$	5·1**	1·1	0·0	2·8
NaCl*$NaNO_2$	1·1	0·8	2·1	0·0
Temp*Temp	94·8*	162·7*	56·3*	162·2*
pH*pH	4·5**	30·6*	41·5*	5·8**
NaCl*NaCl	0·9	5·3**	5·3**	0·0
$NaNO_2$*$NaNO_2$	2·2	0·2	0·0	1·8

F-values are based on type II sum of squares (SAS 1989).
*$P < 0{\cdot}001$; **$0{\cdot}05 < P > 0{\cdot}001$.

The models' fits were also evaluated by comparison of scatter plots of predicted *vs* observed growth kinetics (Fig. 1). Again, the analysis indicated that both the models based on the Gompertz B and M terms and those based on the derived growth kinetics values (i.e. LPD and GT) provided reasonable agreement with the experimental data.

Comparison of the magnitudes of the F-values (based on type II sum of squares) (SAS 1989) associated with the models for B and M, and LPD and GT was used as a means of evaluating the relative importance of the variables and their cross products (Table 3). The majority of the micro-organism's response could be attributed to two of the primary variables, pH and temperature. It should be noted that models based on Ln transformations exclude the no-growth data, which in this case may have diminished the relative importance of NaCl as a primary factor controlling growth. Relatively little effect was associated with the cross product terms, suggesting that the primary variables were largely independent. An exception was a relatively strong pH × NaCl interaction. Adams *et al.* (1991) reported that effects of temperature and pH were independent on the growth of *Y. enterocolitica*. Figure 2 provides three graphic examples of the interactions among pairs of variables on generation time as predicted by model 4 (Table 2).

Comparison of T_{1000} values predicted by the models based on kinetics parameters and Gompertz parameters (Buchanan *et al.* 1989; Bhaduri *et al.* 1994) indicated a high

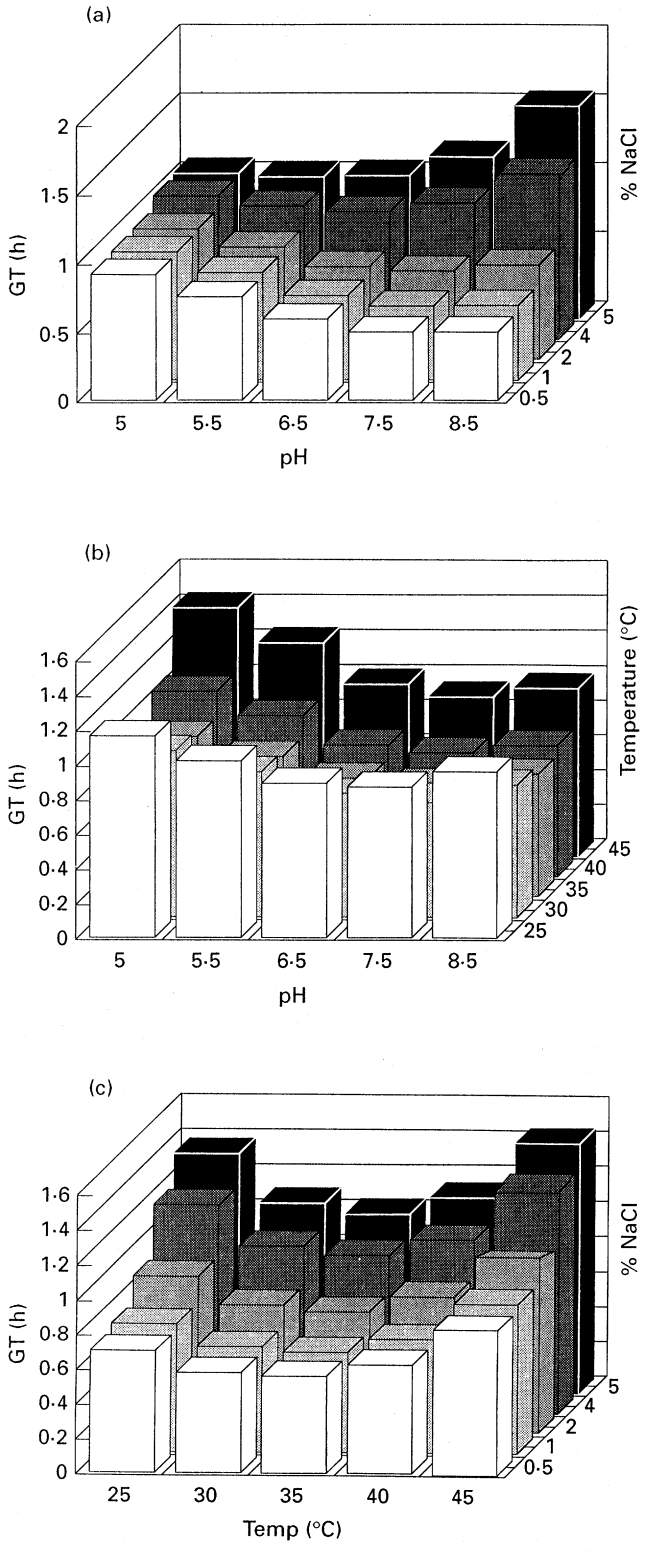

Fig. 2 Examples of characteristics of generation time (GT) of *Yersinia enterocolitica* in Brain Heart Infusion media under various conditions: (a) pH *vs* % NaCl at 37°C; (b) pH *vs* temperature at 2% NaCl; (c) temperature *vs* % NaCl at pH 7·5

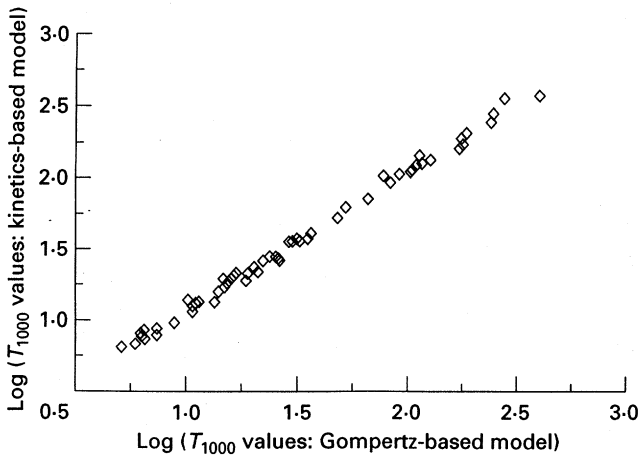

Fig. 3 Comparison of T_{1000} values predicted by kinetics parameters-based and Gompertz parameters-based models

degree of correlation between approaches (Fig. 3). This does not support the suggestion of Garthright (1991) that models based on the derived kinetics terms should be more effective. While the use of kinetics parameters did tend to simplify model generation, the expanded study confirms earlier observations that the approaches are equivalent in models generated using a reasonably good size data set (Bhaduri *et al.* 1994).

Table 4 compares published generation time values for *Y. enterocolitica* in liquid media and meat products with those predicted by model 4 (Table 2) and those of Sutherland and Bayliss (1994) and Baranyi *et al.* (1993). In general, predicted values compare well with each other. However, predicted generation times from the model presented here were typically somewhat more conservative reflecting the desire to provide a model with a margin of safety. The agreement with published values is quite good considering the difficulties in making valid comparisons between predicted and published growth responses, since sufficient experimental detail is not available in the literature. It is important to record all relevant details including at least the pH, temperature and an indication of available water such as NaCl concentration or water activity.

The model reported here is useful because it can demonstrate within seconds the 'worst case', i.e. the most rapid growth of *Y. enterocolitica* that can occur under a given set of combinations of four variables. It should provide food microbiologists with a rapid means of estimating the safety of new or established foods, in relation to the growth of this psychrotrophic pathogen and allow them to more efficiently plan experimentation such as inoculated pack studies. Additional studies on the growth kinetics of *Y. enterocolitica* in a variety of foods are needed to validate the model. It is always recommended that a food processor has

Table 4 Comparison of published and predicted generation times for *Yersinia enterocolitica* in liquid media and meat products

[a]Temperature (°C)	[a]pH	[a]NaCl (%w/v)	[a]Substrate	[a]GT_{publ} (h)	[a]GT_{pred}* (h)	[a]GT_{pred}** (h)	[b]GT_{pred}*** (h)
25	7·2	0·5	BHI	1·00	0·80	0·90	0·60
25	4·6	0·5		2·00	2·00	2·60	1·30
25	6·0	0·5		2·00	0·80	1·00	0·80
25	7·0	0·5		1·00	0·70	0·90	0·70
25	8·0	0·5		1·00	1·50	1·30	0·60
22	7·8	0·5	Buffered BHI	1·00	1·00	1·50	0·80
22	6·6	0·5		1·60	0·90	1·00	0·90
22	5·4	0·5		3·00	1·30	1·70	1·00
22	4·8	0·5		3·00	2·00	3·00	1·40
22	7·4	0·5	Nutrient broth	6·00	1·00	1·00	0·80
37	7·4	0·5		6·00	0·70	0·60	0·50
44	7·4	0·5		6·00	0·90	0·50	0·70
25	5·5	0·5	BHI	1·60	1·00	1·00	0·90
25	6·5	0·5		1·00	0·70	0·90	0·70
25	7·4	0·5		1·00	0·80	1·00	0·60
25	7·1	0·5	Tryptone soya broth (TSB) + yeast extract	2·00	0·80	0·90	0·70
25	6·0	0·5	TSB	1·60	0·80	1·00	0·80
32	8·3	0·1	Phosphate-buffered salts yeast extract medium	0·60	1·00	1·00	0·40
25	8·3	0·1		0·80	1·40	1·60	0·60
22	7·6	0·1	'Basal medium'	1·00	1·00	1·00	0·70
28	7·6	0·1		0·60	0·80	0·80	0·50
32	7·6	0·1		0·50	0·70	0·70	0·40
35	7·6	0·1		0·50	0·70	0·60	0·40
38	7·6	0·1		0·60	0·70	0·60	0·50
30	7·9	0·1		0·50	0·90	0·90	0·40
30	8·6	0·1		0·70	1·60	1·50	0·40
25	6·0	0·5	TSB	1·60	0·80	1·00	0·80
25	5·5	0·5		1·60	1·00	1·00	0·90
30	7·0	0·5	Salts/glucose/ yeast extract broth	1·00	0·60	0·60	0·50
25	7·3	0·5	TSB	1·00	0·80	0·90	0·60
25	5·5–6·5	0·5	Cooked beef	1·00	1·00	1·00	0·90
25	5·5–6·5	0·5	Raw pork	1·00	1·00	1·00	0·90
25	5·5–6·5	0·5	Cooked pork	1·40	1·00	1·00	0·90

[a]GT_{publ} published and [a]GT_{pred} predicted generation times from Sutherland and Bayliss (1994).
* Modified Gompertz model (Sutherland and Bayliss 1994); ** D-model (Baranyi *et al.* 1993); [b]GT_{pred}*** predicted generation time using model 4 (Table 2).

a few tests conducted to demonstrate the validity of any microbial model for a particular food product.

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